

60th Medical Group (AMC), Travis AFB, CA
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
FINAL REPORT SUMMARY

(Please type all information. Use additional pages if necessary.)

PROTOCOL #: FDG20130044A

DATE: 16 July 2015

PROTOCOL TITLE: "Skeletal Muscle Regeneration in a Rat (*Rattus norvegicus*) Model with CorMatrix and Adipose Derived Stem Cells."

PRINCIPAL INVESTIGATOR (PI) / TRAINING COORDINATOR (TC): Major Lucas Neff

DEPARTMENT: General Surgery

PHONE #: 423-5179

INITIAL APPROVAL DATE: 7 October 2013

LAST TRIENNIAL REVISION DATE: 28 August 2014

FUNDING SOURCE:

1. **RECORD OF ANIMAL USAGE:**

Animal Species:	Total # Approved	# Used this FY	Total # Used to Date
<i>Rattus norvegicus</i>	80	0	72

2. **PROTOCOL TYPE / CHARACTERISTICS:** (Check all applicable terms in **EACH** column)

<input type="checkbox"/> Training: Live Animal	<input type="checkbox"/> Medical Readiness	<input type="checkbox"/> Prolonged Restraint
<input type="checkbox"/> Training: non-Live Animal	<input type="checkbox"/> Health Promotion	<input type="checkbox"/> Multiple Survival Surgery
<input checked="" type="checkbox"/> Research: Survival (chronic)	<input type="checkbox"/> Prevention	<input type="checkbox"/> Behavioral Study
<input type="checkbox"/> Research: non-Survival (acute)	<input type="checkbox"/> Utilization Mgt.	<input type="checkbox"/> Adjuvant Use
<input type="checkbox"/> Other ()	<input checked="" type="checkbox"/> Other (Treatment)	<input type="checkbox"/> Biohazard

3. **PROTOCOL PAIN CATEGORY (USDA):** (Check applicable) ☐ C ☒ D ☐ E

4. **PROTOCOL STATUS:**

***Request Protocol Closure:**

☐ Inactive, protocol never initiated

☐ Inactive, protocol initiated but has not/will not be completed

☒ Completed, all approved procedures/animal uses have been completed

5. **Previous Amendments:**

List all amendments made to the protocol.. IF none occurred, state **NONE**. Do not use N/A.

For the Entire Study Chronologically

Amendment Number	Date of Approval	Summary of the Change
1	21 May 2015	Personnel Change

6. **FUNDING STATUS:** Funding allocated: \$ 20,790.00 Funds remaining: \$ 0.00

7. **PROTOCOL PERSONNEL CHANGES:**

Have there been any personnel/staffing changes (PI/CI/AI/TC/Instructor) since the last IACUC approval of protocol, or annual review? ☒ Yes ☐ No

If yes, complete the following sections (Additions/Deletions). For additions, indicate whether or not the IACUC has approved this addition.

ADDITIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, IACUC approval - Yes/No)

Major Lucas Neff PI, IACUC Approval –Yes

DELETIONS: (Include Name, Protocol function - PI/CI/AI/TC/Instructor, Effective date of deletion)

Lt Col Daren Danielson, PI, Effective Date of deletion – 21 May 2015

8. **PROBLEMS / ADVERSE EVENTS:** Identify any problems or adverse events that have affected study progress. Itemize adverse events that have led to unanticipated animal illness, distress, injury, or death; and indicate whether or not these events were reported to the IACUC.

None.

9. **REDUCTION, REFINEMENT, OR REPLACEMENT OF ANIMAL USE:**

REPLACEMENT (ALTERNATIVES): Since the last IACUC approval, have alternatives to animal use become available that could be substituted in this protocol without adversely affecting study or training objectives?

No.

REFINEMENT: Since the last IACUC approval, have any study refinements been implemented to reduce the degree of pain or distress experienced by study animals, or have animals of lower phylogenetic status or sentience been identified as potential study/training models in this protocol?

No.

REDUCTION: Since the last IACUC approval, have any methods been identified to reduce the number of live animals used in this protocol?

No.

10. **PUBLICATIONS / PRESENTATIONS:** (List any scientific publications and/or presentations that have resulted from this protocol. Include pending/scheduled publications or presentations).

No.

11. **Were the protocol objectives met, and how will the outcome or training benefit the DoD/USAF?**

Yes. This study provided evidence that extracellular matrix made from swine small intestinal submucosa does not provide a regenerative scaffold for growth of skeletal muscle after volumetric muscle loss.

12. **PROTOCOL OUTCOME SUMMARY:** (Please provide, in "ABSTRACT" format, a summary of the protocol objectives, materials and methods, results - include tables/figures, and conclusions/applications.)

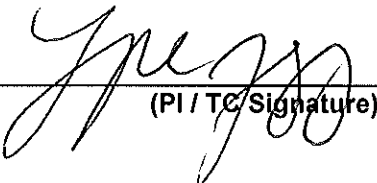
Introduction: The increasing use of explosive devices has led to a dramatic increase in traumatic injury accompanied by severe tissue trauma and volumetric muscle loss, with a clinical need for enhanced repair. Extracellular matrix (ECM) shows promise to regenerate skeletal muscle, opening a therapeutic avenue for patients with devastating traumatic injury and irreparable volumetric muscle loss.

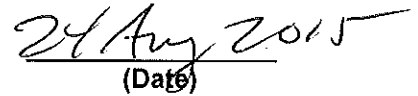
Methods: A 7mm full thickness volumetric muscle loss defect was surgically created under anesthesia in the medial head of the rat gastrocnemius muscle, utilizing a specially designed muscle biopsy clamp. ECM derived from porcine small intestine was evaluated for repair of the defect, in both sheet and particulate forms. Defects in the control group were left unrepaired. Rats were sacrificed at 3, 6, and 10 months for histologic evaluation and immunohistochemistry to evaluate muscle healing. Isometric functional testing was implemented prior to euthanasia

at 10 months to further evaluate healing at later time points. Instrumentation for surgery was designed and fabricated for specific study use, as was hardware and apparatus setup for function testing.

Results: The animal model described here was shown to be reproducible and the specially designed muscle biopsy clamp helped to standardize the size of muscle defects. ECM sheet and emulsified matrix were successfully implanted to bridge the gap in study animals. No visible wound related complications, gait deficits or pain were observed in animals post-operatively. At 3 and 6 months post-operatively, there was evidence of angiogenesis, but it was not overly apparent that the use of the matrix fostered muscle regeneration across the large gap. A minimal degree of inflammation was observed in the regenerated tissue within the ECM tube along with a compensatory increase in soleus muscle mass in response to defect creation. There was no significant difference in force generation among groups at 10 months post operatively.

Conclusion: Herein, we present a novel model for evaluation of muscle regeneration. In collectively evaluating results from histologic analyses, muscle morphometry, and functional study outcomes, data seems to indicate that there is no difference among groups or time points; and there is no evidence of muscle regeneration across the gap area in any group. While the full thickness defect recapitulates the nature of severe clinical injury, the regenerative strategies explored in this study were insufficient to return substantial muscle function after 10 months. This model was validated in the first phase of study and currently, work is underway to further assess the ability of the matrix to work in concert with adipose derived stem cells for further augmentation of healing.


(PI / TC Signature)


(Date)

Attachments:

Attachment 1: Defense Technical Information Center (DTIC) Abstract Submission (Mandatory)

Attachment 1

Defense Technical Information Center (DTIC) Abstract Submission

This abstract requires a brief (no more than 200 words) factual summary of the most significant information in the following format: Objectives, Methods, Results, and Conclusion.

Introduction: Extracellular matrix (ECM) shows promise to regenerate skeletal muscle in patients with volumetric muscle loss.

Methods: A full thickness muscle defect was surgically in the medial head of the rat gastrocnemius muscle,. ECM derived from porcine small intestine was used to repair the defect. Rats were sacrificed at 3, 6, and 10 months to evaluate muscle healing.

Results: No visible wound related complications, gait deficits, or pain were observed in animals post-operatively. At 3 and 6 months there was evidence of angiogenesis, but the matrix did not stimulate muscle regeneration across the large gap. There was no significant difference in force generation among groups at 10 months post operatively.

Conclusion: Results from histologic analyses, muscle morphometry, and functional testing, data seem to indicate that there is no difference among groups or time points; and there is no evidence of muscle regeneration across the gap area in any group.

Grant Number: _____

From: _____

****If you utilized an external grant, please provide Grant # and where the grant came from. Thank you.**